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The genetics of spot pattern characters in the meadow brown butterfly *Maniola jurtina* (Lepidoptera: Satyrinae)

Paul M. Brakefield* and Arie J. van Noordwijk†

Department of Population and Evolutionary Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The control of spot pattern variation on both hindwing and forewing in 16 F_1 broods of *Maniola jurtina* is analysed. Substantial additive genetic variance was demonstrated for each of the spot pattern characters. For example, estimates for heritability of hindwing spot-number were $0{\cdot}66 \pm 0{\cdot}11$ in males and $0{\cdot}89 \pm 0{\cdot}11$ in females. These estimates were essentially the same when four broods with extremes of low or high mortality were excluded from the analysis. The hindwing traits showed a consistent pattern of a closer resemblance of offspring to the same sex than to the opposite sex parent. This can also be expressed in a genetic correlation between the same trait in the two sexes that is (much) less than 1·0. Further analysis strongly suggested that this follows from a partially sex-dependent expression of some of the individual spots. In constrast to the hindwing traits, there may be a maternal effect in the inheritance of the forewing "eyespot". The fact that relative differences in spot-number between populations tended to be maintained when wild larvae were reared in the laboratory and experiments which showed no significant influence of varying temperature or relative humidity during periods of the pupal stage on spot phenotype suggest that the estimates of heritability are likely to be close to those which pertain in natural conditions. Analysis of data for relative hindwing spot-size in the broods provides some support for a threshold model for spot development.

INTRODUCTION

The meadow brown butterfly *Maniola jurtina* (L.) exhibits considerable quantitative variation in the submarginal spot pattern of its wings. For this reason the species has been extensively studied by ecological geneticists (see reviews by Ford, 1975 and Brakefield, 1984). These studies have concentrated on describing the spatial and temporal dynamics of the different phenotypes within populations and on pointing out the selective forces that might be operating on these variations. Although the crucial importance of understanding the genetic control of the expression of spotting characteristics has always been acknowledged, practical problems have hitherto prevented obtaining reliable estimates of the heritability of these traits. McWhirter (1969) obtained very limited data on the heritability of the number of spots on the hindwings. He reared four broods (total $n = 89$) from parents caught *in copula* or raised from fifth instar larvae collected on the Isles of Scilly in southwest England. The paucity of these data provides a major reason for casting doubt on McWhirter's estimates. This applies particularly to his finding that whilst the heritability in females was very high, there was only a very low heritability in males. This is unfortunate because such a difference in the pattern of inheritance between the sexes would fit well with the hypothesis that the forces of natural selection which act on the spot pattern in males and females differ (Handford, 1973; Brakefield, 1982; 1984). This hypothesis is based on observed differences in the expression of the spot pattern and in adult behaviour between the sexes. For example, males show smaller forewing "eyespots", tend to have more hindwing spots and are more active than females. Here, we report on more extensive breeding data that provide more reliable heritability estimates for a series of spot pattern characters on both hindwing and forewing. Analysis of some of the data in several different ways demonstrates how a partially sex-dependent

* *Present address*: Department of Biological Sciences, Washington Singer Laboratories, University of Exeter, Perry Road, Exeter EX4 4QG, U.K.

† *Present address*: Zoologisches Institut der Universität Basel, Rheinsprung 9, CH 4051 Basel, Switzerland.

expression of certain traits is reflected in the analyses. The spot pattern characters are also of interest as an example of traits where both quantitative genetic analyses and those based on presence or absence of discrete traits can be merged.

It is important that data for heritability estimates are collected under environmental conditions that are relevant to the natural environment about which we wish to draw inferences. There have been a few field studies on birds where genetic variation for morphological and reproductive traits has been demonstrated (see refs. in van Noordwijk, 1983). Unfortunately, it is impossible to collect the necessary data on family relationships for butterflies in the field. However, our data were collected under conditions that were in some respects intermediate between the natural environment and a rigorously controlled laboratory environment. Some additional small experiments showed little effect of varying temperature during the most sensitive period for pattern determination. We believe, therefore, that the heritability estimates presented in this paper are relevant for the natural conditions. Evidence is also presented that the parent–offspring relationship for hindwing spot-number is independent of variability between broods in mortality.

MATERIALS AND METHODS

(i) *Source population*

Eggs were obtained in 1979 from 30 females collected from a population at Oudemirdum in the province of Friesland, The Netherlands. The adults ($n > 300$) reared from these eggs were used to set up the 16 broods analysed in this paper. The parental and F_1 generations were raised under similar conditions.

(ii) *Rearing methods*

M. jurtina can easily be paired in net cages and females will lay readily in small plastic boxes covered with a cotton net. Young larvae were raised on seedling grasses from a lawn grass seed mix sown in 20 cm pots. Mid to late instar larvae were fed on grass plants (mainly *Poa annua*) transplanted from outside into 45 cm square boxes. Broods were kept in an unheated laboratory with reduced temperature fluctuations in comparison to outdoors. During the pupation period for the broods (59 days) the mean (±S.D.) daily maximum and minimum temperatures were 20·5±4·6°C and 14·8±3·1°C respectively.

Counts of the larvae in the broods were made at the first or early second instar stage to examine the level of mortality. The causes of mortality were not investigated but there was no indication of any outbreaks of microbial disease. Such infections have been characteristic of stocks from the British mainland (McWhirter, 1965; Brakefield, 1979, unpublished observations). Our broods may have been protected from infection by the UV component of the "Gro-lux" strip lamps under which they were reared (with natural day length) or, alternatively, the original stock may have been free of pathogens.

(iii) *Description of traits*

The underwing spot pattern is illustrated in fig. 1. The small hindwing spots are not usually expressed on the upperwing surface. On the underwing, spots may be present or absent at each of six positions.

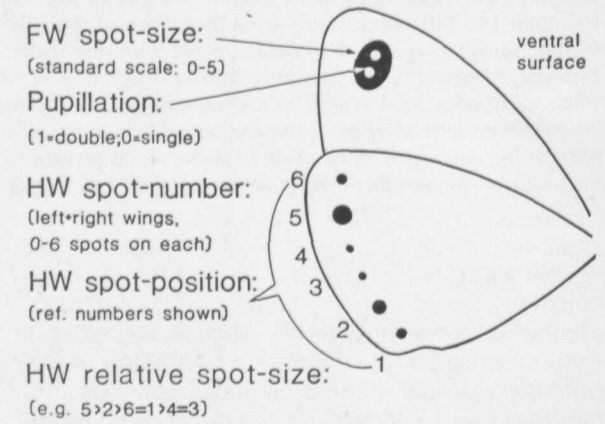

Figure 1 Spot pattern characters in *Maniola jurtina*.

The size of the spots varies continuously (Brakefield, 1979; 1984). The spot at position 4 (spot 4) is not found in many populations but was present in the study material. The range in spot-number is therefore from 0 to 6 on each wing. The spots are usually encountered in a limited series of different spot-position combinations (see McWhirter and Creed, 1971). The hindwing spot characters recorded on the left wings were (1) spot-number, (2) spot-position and (3) the relative size of the spots as assessed by visual inspection (see fig. 1). The first two traits were also recorded on right wings to detect examples of bilateral asymmetry. The spot-number data analysed in this paper are the totals for both hindwings of each butterfly.

The breeding material was high spotted. An advantage of this is that the frequency distribution of spot-number is less skewed than for the many low spotted populations (see *e.g.*, Ford, 1975).

A preliminary analysis of the size of the forewing "eyespot" was made. Butterflies were compared to a set of size standards on a scale from 0 to 5 in each sex. The number of white pupils in this spot was also recorded (see fig. 1).

(iv) *Genetic analyses*

Only elementary methods were used (after Falconer, 1981). In studies, like this one, on material from heterogeneous environments (in this case over time) it is important to check explicitly for familial resemblance caused by sharing of environments by relatives. We will therefore present some elementary statistics about our data in the results section. Several non-standard procedures will be explained together with their results.

(v) *Tests of environmental effects*

The pupae of one F_1 brood were used to investigate the effect of chilling on the development of hindwing spot pattern which occurs during the early part of the period of pupation (see Nijhout, 1980; Robertson, 1981). Pupae were assigned at random to unheated laboratory conditions or to treated groups which were cooled at $2 \pm 1°C$ for 24 or 48 hours at different ages in relation to the time of pupation.

In other experiments, collections of wild larvae from three populations were reared in the laboratory and the pupae kept in different temperature or relative humidity regimes. The hindwing spot-number variation of the reared adults is also compared with that in samples of flying adults from the same populations. Full details of these experiments are given in Brakefield (1979).

RESULTS

The conclusions reached about the statistical significance of items in the following analyses should be interpreted with caution since they are based on the variance present within this sample of only 16 broods. This is emphasised by the wide variation in family size (mean ±S.E.: 83·75 ± 19·2; range: 8–260). We consider the breeding data as a starting point for hypothesis generation rather than for testing hypotheses. Additional data will be required for adequate tests. The raw data for hindwing spot-number are presented in Brakefield (1984) together with illustrations of samples from six families.

(i) *Hindwing spot-number*

Spot-number is the most direct way of examining variation in hindwing spot-pattern. The regression of mean spot-numbers for all male and for all female offspring in a brood on those of the male, the female and the mid-parent yields initial estimates of heritability (table 1). All these six estimates are high, significantly different from zero and not significantly different from each other.

Table 1 Heritability estimates (±S.E.) for hindwing spot-number in *Maniola jurtina* based on regression on single parent and mid-parent values; untransformed data. All estimates are significantly different from 0. The correlation between parental spot-numbers was 0·09 and the genetic correlation between sexes was 0·75

	Male parent	Female parent	Mid-parent
Male offspring	0·88 ± 0·21	0·58 ± 0·26	0·66 ± 0·11
Female offspring	0·85 ± 0·32	1·08 ± 0·25	0·89 ± 0·11

Male offspring, however, apparently resemble their male parent more closely than their female parent, and female offspring are apparently more like their female than their male parent. This could be caused by different scaling properties of the traits in the two sexes, or by a different pattern of inheritance, *e.g.*, sexual differences in the expression of the traits. The first of these possibilities can be examined further.

Two approaches were taken, firstly by applying transformations to or using selections from the data and recalculating the heritabilities to see if the pattern is sensitive to such manipulations; and secondly by examining the correlations between several mean and variance parameters. The first approach, using log transformation, square root transformation, excluding small broods or excluding individuals that show bilateral asymmetry has no noticeable effect on the estimates. Similarly, there is no difference between estimates that take into account the variability in family size and those from simple unweighted regressions (see Brakefield, 1984).

The second approach is summarized in table 2. Eight correlations are given for both untransformed data and the square roots of spot-numbers. The mean and the variance in the offspring of one

sex are each highly correlated with the corresponding statistic for the other sex. The correlations between means and variances are similar and positive for each sex, though they do not reach formal significance for our small number of broods. The effect of applying transformations suggests that there is a scaling effect. The optimal transformation is likely to be weaker than the square root one. The correlations in table 2 also indicate a tendency for offspring (of both sexes) to be more variable if the difference in phenotype between the parents is greater. This tendency is only apparent from correlations with the absolute value of the difference between the parents and not from the signed difference, indicating that there is no difference between the male being more or being less spotted than the female. The fact that the first two correlations in the table are not sensitive to scaling suggests that scaling artifacts cannot explain the apparent pattern of sex-dependent expression of spot-number.

Table 2 Correlation coefficients for mean and variance statistics of hindwing spot-number in offspring broods of *Maniola jurtina*. Coefficients for untransformed data and after square root transformation are compared by calculating a normal deviate Z. Abbreviations: M_m, M_f = mean of male, female offspring. V_m, V_f = variance in male, female offspring. D_p = difference in parental values (male value − female value). $|D_p|$ = absolute difference in parental values

Comparison	Raw data	Transformed data	Z
$M_m - M_f$	0·83‡	0·82‡	0·08
$V_m - V_f$	0·64†	0·51*	0·50
$M_m - V_m$	0·47§	−0·11	1·58
$M_f - V_f$	0·33	−0·38	1·89†
$V_m - D_p$	−0·27	0·09	0·94
$V_f - D_p$	0·09	−0·16	0·64
$V_m - \|D_p\|$	0·20	0·25	0·13
$V_f - \|D_p\|$	0·37	0·19	0·50

* $P < 0{\cdot}05$; † $P < 0{\cdot}01$; ‡ $P < 0{\cdot}001$; § P approaches 0·05.

The intra-class correlations on which estimates of heritability of spot-number can be based are 0·39 for males and 0·44 for females. These yield estimates of 0·78 and 0·88, repectively for the untransformed data (cf. McWhirter, 1969; and see Falconer, 1981, p. 135). These values are similar to the estimates for heritability based on parent-offspring regression (table 1).

(ii) *Hindwing spot-position*

The variation in hindwing spot pattern can also be examined by calculating a mean position for all spots that are present in an individual using the reference numbers for the spots as shown in fig. 1. There is evidence that in natural populations mean spot-position is largely independent of mean spot-number (McWhirter and Creed, 1971; Brakefield, 1984). Only if nearly all spots are present in an individual is the mean position constrained within narrow limits. Table 3 shows that this trait is heritable. Again, there is a closer resemblance between parents and offspring of the same sex than those of different sex. In this case the genetic correlation between the two sexes is only 0·40, which suggests that the expression of specific spots is sex-dependent. This can be tested by analysing the frequency of the presence of individual spots in the offspring of each sex in the same brood.

Table 3 Heritability estimates (±S.E.) for hindwing spot-position in *Maniola jurtina* based on regression on single parent and mid-parent values; unspotted butterflies are excluded. Estimates which are significantly different from 0 are indicated by asterisks. The correlation between parental values was 0·01 and the genetic correlation between sexes was 0·40

	Male parent	Female parent	Mid-parent
Male offspring	0·53 ± 0·17*	0·24 ± 0·16	0·35 ± 0·10*
Female offspring	0·27 ± 0·40	0·77 ± 0·25*	0·57 ± 0·20*

(iii) *Presence of individual spots*

The data from the ten broods which contained at least 20 offspring of each sex were used in the analysis of individual spots. Three of the six spots are of no interest for this purpose. Spot 5 was present in all parents and in nearly all offspring; spot 4 was absent in all parents and was present in the offspring of only two broods; and spot 1 was present in only six male and one female parent. In none of these cases was the difference in frequency between the sexes significant. Significant differences between sexes were, however, found in the other three spots.

Spot 6 was present in four male and in seven female parents and, with one exception, was found more frequently in female offspring than in male offspring in the same brood. In the only brood where the spot was present in the male, but absent in the female parent, the frequency was higher in male offspring than in female offspring, although this difference was not significant. In three broods the frequencies in female offspring were significantly higher than in males (table 4). This spot, therefore, may be characterized as "female".

Spots 2 and 3 were found more often in males than in females and in many broods the frequencies in the offspring were significantly different between the sexes (table 4). For spot 2 nothing can be discerned about the genetics because this spot was present in all parents. For spot 3 there were two broods where the spot was absent in both parents and in which the frequencies were low and similar in each sex. In two broods the spot was present in the female parent only. In one of these the frequency was somewhat higher in the female offspring, whilst in the other the frequency was significantly higher in the male offspring (table 4). In all six broods with the spot present in the male parent only, the frequency was significantly higher in the male offspring. These two spots may, therefore, be referred to as typically "male".

Table 4 Frequency of spot-presence in male and female offspring in relation to spot-presence in the parents. Frequencies are compared by the *G*-test, assuming equal frequencies in both sexes. Significant departures are given for broods with $n \geqq 20$ for each sex

	Parents		% Frequency		
	male	female	males	females	*P* value (*G*-test)
Spot 6	+	+	68	79·5	0·009
	+	+	49	81	0·003
	−	+	46	71	0·023
Spot 3	+	−	23	1·5	0·028
	+	−	83	43	10^{-9}
	+	−	49	12	10^{-6}
	+	−	79	46	0·002
	+	−	50	6	10^{-5}
	+	−	64	36	0·019
	−	+	80	37	0·002
Spot 2	+	+	98	23·5	10^{-6}
	+	+	100	80·5	10^{-6}
	+	+	100	70	10^{-6}
	+	+	100	95·5	0·026
	+	+	100	84	0·002
	+	+	100	51·5	10^{-6}
	+	+	100	63	10^{-4}
	+	+	88·5	52	0·005

Apart from the six small broods and the three spots with low variability, we find 18 (out of 30) significant deviations from equal spot frequencies in both sexes within broods. The existence of a typically female (6) and two typically male spots (2 and 3) will cause a greater resemblance to the same sex parent in respect of both spot-number and spot-position.

(iv) *Relative hindwing spot-size*

The data on relative spot-size are rather unsatisfactory since there is considerable variability in absolute spot-size between individuals that are scored as being the same. The data are nevertheless interesting because of the relationship between spot-presence and spot-size. In general spot-number is highly correlated with total hindwing spot-area and increases in *average* spot-size with increasing spot-number are small (Brakefield, 1984; 1979).

The preceding results strongly suggest that the inheritance of hindwing spot pattern could be described in terms of a dosage model with a series of thresholds below which each of the individual spots are absent (see *e.g.*, Nijhout, 1978; 1980). In such a model one expects that above such a threshold a high concentration of the morphometric substance or morphogen will result in a large spot. It then follows that there will be a correlation between spot-size in the parents and the frequency of spot-presence in the offspring. It is noteworthy that in our bred material as a whole there is indeed a close correspondence in the hierarchies of overall spot-presence and relative spot-size. Thus spot 5 was almost always present and was nearly always the largest spot, followed by spot 2, while spot 1 was usually the smallest spot and occurred at the lowest frequencies. A similar conclusion was reached from a morphometric analysis of thirteen populations (Brakefield, 1979; 1984).

The analysis of this relationship by parent–offspring correlation is more complex. Table 5 gives the correlations between the presence and relative size for each spot and each sex. The top left columns show high positive correlations between spot-presence in the parent and spot-frequency in the offspring. In the bottom right part of the table there is no clear pattern in the correlations between relative spot-size in the parent and relative spot-size in the offspring, especially when the analysis is restricted to those spots that are present. The top right part of the table indicates that a strong correlation between size in the parents and frequency in the offspring is found only for spot 5. This correlation occurs in both sexes.

Conversely, presence or absence in the parents is correlated with the relative size index in the offspring most clearly for spot 1 (bottom left part of table 5). Therefore clear relationships are found for the largest and for the smallest spots, but not for the intermediate ones. The extremes are precisely those spots where the relative size index is most accurate in reflecting the absolute size. Thus

Table 5 Correlation coefficients between frequency of spot-presence and indices of relative spot-size for individual hindwing spots in parents and offspring of the same sex. The number of broods is 16 unless indicated otherwise in parentheses. (a) frequency in offspring, (b) spot-size in offspring

	presence/absence		Parents relative size[1]		relative size[2]	
	males	females	males	females	males	females
(a)						
Spot 6	0·58*	0·94†	0·59*	0·72‡	0·29 (6)	−0·17 (11)
5	—	—	0·61*	0·83‡	0·61*	0·83‡
3	0·49	0·19	0·51*	0·18	0·20 (8)	−0·34 (3)
2	—	0·71†	0·23	0·67†	0·23	0·21 (13)
1	0·53*	0·90‡	0·60*	0·90‡	0·37 (8)	— (1)
(b)						
Spot 6	0·08	0·20	0·08	0·28	0·10 (6)	0·60* (11)
5	—	—	−0·19	−0·07	−0·19	−0·07
3	−0·05	0·06	−0·04	0·04	−0·23 (8)	−1·00 (3)
2	—	0·60*	0·32	0·56*	0·32	0·20 (13)
1	0·75‡	0·56*	0·72†	0·56*	0·24 (8)	— (1)

* $P<0·05$; † $P<0·01$; ‡ $P<0·001$.
[1] Including spot-absence (= size 0).
[2] Excluding spot-absence.

our conclusion is that a correlation is evident in cases where the spot-size records were reasonably accurate.

(v) *Forewing spot-size*

The heritability estimates for forewing spot-size are high and the genetic correlation between the sexes is much higher than for the previous two traits (table 6). The heritability estimates based on a comparison with the female parent are higher than those based on the male parent. This is not due to a difference in the parental variances between the sexes but rather to differences in the covariances. There is therefore some indication of a maternal effect. It would be interesting to see whether a maternal effect acts through an effect of egg-size on wing-size. This would require morphometric data that were not collected.

Table 6 Heritability estimates (±S.E.) for forewing spot-size in *Maniola jurtina* based on regression on single parent and mid-parent values. Estimates which are significantly different from 0 are indicated by asterisks. The correlation between parental values was −0·21 and the genetic correlation between sexes was 1·09

	Male parent	Female parent	Mid-parent
Male offspring	0·40 ± 0·36	0·85 ± 0·29*	0·80 ± 0·21*
Female offspring	0·27 ± 0·32	0·66 ± 0·27*	0·59 ± 0·20*

(vi) *Pupillation of forewing spot*

The frequency of forewing spots with two pupils is generally much higher in females than in males. The study population exhibited a high frequency of bipupillation compared to samples from the British Isles (Brakefield, 1979). Table 7 gives the frequencies of bipupillation in the offspring, grouped according to the parental phenotypes.

Table 7 Bipupillation of the forewing spot in *Maniola jurtina*. Both wings in the offspring were scored, and frequency data are grouped according to parental phenotypes

Number of broods	Parental phenotype		Bipupillation in offspring			
			males		females	
	male	female	*n*	%	*n*	%
7	−	−	712	6·5	718	72·7
7	−	+	302	17·2	322	84·5
2	+	+	356	67·1	280	99·3

There is clearly a positive relationship in that the frequencies of bipupillation are higher, especially in male offspring, when the trait is present in the parents, but further data are needed before more sophisticated analyses, such as a heritability estimate based on a probit transformation, would be justified.

Table 8 The effect on hindwing spot-number and spot-position of cooling pupae of *Maniola jurtina* (from brood 14) during different phases of pupation. The larvae were divided into (a) early and (b) late pupating groups. Cooling was at 2 ± 1°C with controls in an unheated laboratory

		Males			Females		
Treatment*	Duration of cooling (h)	*n*	spot-no.† $\bar{x} \pm 95\%$ CL	mean spot-posn.	*n*	spot-no.† $\bar{x} \pm 95\%$ CL	mean spot-posn.
(a)							
1	24	21	7·67 ± 0·32	3·56	6	7·33 ± 0·84	3·78
2	24	15	7·73 ± 0·36	3·48	3	7·67 ± 0·67	3·71
1+2	24	36	7·69 ± 0·24	3·53	9	7·44 ± 0·59	3·76
controls	0	36	7·50 ± 0·34	3·56	5	6·80 ± 0·98	3·80
(b)							
3	48	9	6·44 ± 1·30	3·64	17	6·41 ± 0·96	4·08
4	48	7	6·57 ± 1·14	3·49	17	6·47 ± 0·80	4·04
3+4	48	16	6·50 ± 0·86	3·57	34	6·44 ± 0·62	4·06
controls	0	18	6·89 ± 0·67	3·74	44	5·77 ± 0·52	4·30

* Treatments 1–4 began at increasing ages in relation to time of pupation: 1 = 0–15 min.; 2 = 15 min–12 h; 3 = phase with yellowing of wing cases; 4 = phase just showing wing pigmentation especially of forewing "eyespot".
† Variance statistics are given since the frequency distributions are approximately normal. Within (a) and (b) none of the differences between the means for males or females is significant at $P < 0{\cdot}05$.

(vii) *Environmental effects on hindwing spot pattern*

The results of the experiments with one brood on the effects of a period of chilling of the pupae are given in table 8. There is little indication of an influence of cold temperature on hindwing spot-number or spot-position. The treated groups of pupae included those subjected to chilling immediately after or within twelve hours of pupation. This early part of the pupal stage corresponds with that for which experiments with various species of butterfly have shown sensitivity of wing pattern elements to temperature shocks on the pupae (Høegh-Guldberg and Hansen, 1977 and refs. therein). In many cases the temperature sensitive period does not extend beyond about 48 hours after pupation though this depends on the character studied and the rearing temperature (see *e.g.*, Nijhout, 1980). Høegh-Guldberg and Hansen's experiments on the lycaenid *Aricia artaxerxes* showed that periods of chilling for 9–12 hours close to the time of pupation produced a lower mean spot-number and a number of aberrant forms. Our experiments show no evidence for a comparable sensitive period in *M. jurtina* or for one occurring later in the pupal stage.

The results of rearing pupae of *M. jurtina* raised from collections of wild larvae at different temperatures or relative humidities similarly show no effect of these variables on hindwing spot-number variation (table 9). The three source populations for the larvae exhibited widely differing frequency distributions for spot-number with examples of both low (Cramond Is., E. Scotland) and high (Buckley, N. Wales and St Martin's, Isles of Scilly, S.W. England) mean spot-numbers (see also Brakefield, 1979). These relative differences tended to be maintained in the reared material. However, for St Martin's Is. there is a highly significant difference between the reared and the flying adults (table 9). The latter difference is interpreted as evidence for powerful directional selection acting during pre-adult development in this population (Brakefield, 1984). The larvae from St. Martin's Is. were obtained at an earlier stage of development than those from the other populations.

DISCUSSION

Our estimates of heritability will only be reliable if the mortality occurring in the broods was non-selective with respect to the spot characters. The mean mortality (±S.E.) in a brood measured from first or second instar larvae to adults was 42·7 ± 3·2 per cent. The overall mortality was 38·5 per cent. Thus there was substantial mortality which probably amounted to about 45 per cent over the whole generation time of nearly a year. There were highly significant differences among the 16 broods in the measured mortality ($\chi^2 = 186{\cdot}3$, 15 df, $P < 0{\cdot}001$). Some indication of whether this mortality is selective can be obtained by examining its relationships to hindwing spot-number variation in the parental and F_1 generations. For example, if mortality was

Table 9 The effect of varying temperature or relative humidity during the pupal stage on hindwing spot-number in adults for stocks of *Maniola jurtina* collected as mid or late instar larvae in the populations indicated. The frequency distributions for spot-number in the subsamples are compared by heterogeneity chi-square. The spot frequency distributions in the total reared adults and the individuals collected as adults are also compared by chi-square for each population

		Males			Females		
Population	Treatment	*n*	mean spot-no.	chi-square (df)	*n*	mean spot-no.	chi-square (df)
Buckley 1975	25–27°C	7	5·14		27	2·96	3.09
	21–23°C	5	6·80	—	17	3·76	(6)
	7–12°C	9	4·44		17	2·35	
	total reared	21	5·24	0·71	61	3·02	4·26
	wild caught	57	5·26	(4)	43	3·44	(4)
Cramond Is. 1975	25–27°C	16	4·00	1·17	29	1·17	6·54
	21–23°C	35	3·83	(4)	28	1·29	(4)
	7–12°C	12	3·50		22	1·36	
	total reared	63	3·81	4·65	79	1·27	2·71
	wild caught	62	4·06	(4)	87	1·68	(3)
St Martin's Is. 1976–77	23–25°C	78	7·38	2·39	57	3·40	2·14
	11–14°C	70	7·23	(3)	57	3·65	(4)
	90–100% r.h.	30	7·67	0·95	23	3·22	4·75
	32–37% r.h.	38	7·47	(3)	23	4·70	(2)
	total reared	216	7·39	32·19‡	160	3·65	14·47†
	wild caught	32	5·00	(3)	69	2·49	(4)

† $P<0{\cdot}01$; ‡ $P<0{\cdot}001$.

heaviest for the higher or lower spotted phenotypes then non-random relationships between mortality and spot variation would be expected. The correlations between percentage mortality and the mid-parent and mid-offspring values are not significant ($r=-0{\cdot}38$ and $-0{\cdot}245$ respectively, $P>0{\cdot}1$). Examination of the mortality data shows that there are two broods with fewer than 31 per cent of deaths (21 and 23·5 per cent) and two with more than 52 per cent (62 and 70 per cent). The heterogeneity in mortality in the remaining broods is smaller, although it remains significant ($\chi^2=27{\cdot}3$, 11 df, $P<0{\cdot}01$). The correlation coefficients between percentage mortality and mid-parent and mid-offspring values when the four extreme broods are excluded are $+0{\cdot}01$ and $-0{\cdot}03$ respectively. Estimates of heritability (±S.E.) of spot-number obtained using regression on mid-parent values for the 12 broods are closely similar to those given in table 1 for all data (males, $0{\cdot}675\pm0{\cdot}26$; females, $0{\cdot}94\pm0{\cdot}27$ with $P>0{\cdot}1$ for each comparison). These observations thus provide no evidence that our estimates are influenced by selective mortality during development.

Our data are derived from a breeding experiment in the laboratory and it is therefore legitimate to question the extrapolation of our heritability estimates to the field conditions. Two lines of argument can be raised which suggest that our estimates are likely to be in reasonable agreement with what would be found under natural conditions. Firstly, when larvae collected in the field are reared in the laboratory, relative differences in hindwing spot-number tend to be maintained (table 9 and see Dowdeswell, 1961; 1962; Brakefield, 1984). The exceptionally high spotting of the population at Oudemirdum is also found in our bred material (Brakefield, 1984). Secondly, the experiments in which temperature or relative humidity was varied during pupal development showed no significant effects on the spot phenotype (tables 8 and 9; see also Brakefield, 1979).

Numerous investigations on various species of butterflies have used morphometric techniques to analyse spot and other wing pattern characteristics which exhibit continuous variation (see review by Brakefield, 1984). Such studies have frequently demonstrated differences between populations and sometimes changes with time within populations. These results and similar ones in other taxonomic groups have led many workers to advance interpretations in terms of natural selection. In doing so, it is of course critical that evidence for a genetic component in the control of the characters is obtained. This has rarely been done. Exceptions in butterflies are the study of geographic variation in wing pattern in *Heteronympha merope* (Pearse and Murray, 1981; 1982) and a study of variation in the number of raylets in *Heliconius erato* (Pansera and Araújo, 1983). The presence of

genetic variation if phenotypic variation is observed cannot be taken for granted. A recent study by Arthur (1982) of shell shape in the snail *Lymnaea stagnalis* provides a powerful illustration of the need to consider alternative explanations for the observed phenotypic variation and the need to test them experimentally. This study of *M. jurtina* provides an example where interpretations in terms of selection have a sound basis in the substantial amount of additive genetic variance for each of the spot pattern characters that were analysed.

Our results provide no support for McWhirter's (1969) preliminary findings of a heritability for hindwing spot-number that was high in females, but low in males, though they do support the hypothesis that the expression of at least some individual spots is partially sex-dependent. This could be confirmed by practising disruptive selection on the offspring of each sex within broods for the expression of particular spot combinations. It would also be of interest to determine whether artificial selection applied in opposing directions on the sexes would produce a divergence in spot-number. In many genetic experiments artificial selection on a character in one sex only has produced a strong response in the other sex as well (see Lande, 1980 for a discussion). The observation that the hindwing spots have a pattern of expression that is partially sex-dependent due largely to the behaviour of certain spots is of particular interest since it is consistent with a model to account for the variability in spot patterns in natural populations (Brakefield, 1984; see also Brakefield and Larsen, 1984). This model predicts that visual selection by predators operates differently on the two sexes and, less strongly, also operates disruptively within the sexes. These differences in selection are associated with differences in the adult behaviour and with different resource requirements of males and females (Brakefield, 1982). The marked sexual dimorphism in the size of the forewing "eyespot" is an important component of the model. Significant differences in forewing spot-size in each sex have been found between populations within a region of Scotland (Brakefield, 1984). This study also suggests that variability in this character has a substantial genetic component.

The present study and the morphometric analysis performed by Brakefield (1979; 1984) lend support to the notion that a threshold system is involved in the determination of spot phenotype (*cf.* Nijhout, 1978; 1980). Our findings of parent–offspring relationships between an index of spot-size and spot-frequency for some of the hindwing spots are what one expects if the same morphogenetic substance has an effect on spot-size above the threshold for spot-presence.

There are numerous examples of apparently powerful selection acting on spot-number variation in *M. jurtina* (for reviews see Ford, 1975; Brakefield, 1984). In view of the high heritability of this trait our results emphasise the caution necessary in interpreting theoretical predictions that heritability is likely to be low for high fitness traits (see discussion in van Noordwijk, 1984). The estimation of heritabilities in a single population is a first step that can be continued in several directions. The common theme of these different approaches is the integrated study of the (architecture of) genetic variation and the natural selection operating on these traits. The contribution of this study is to give heritability estimates and to generate hypotheses about the genetic architecture, especially a sex-dependence in the expression of spot pattern genotypes, in a species for which hitherto the investigations have been concerned mainly with the study of natural selection.

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